J. Food Sci. Nutr.
Vol. 6, No. 1, p. 51~56 (2001)

Effects of Antioxidant Nutrient Supplementation on the Lipid Peroxidation and Antioxidative Enzyme Activities in Patients with Coronary Heart Disease

Hyojee Joung†, Byung Yeol Chun*, Young Sun Choi, Sueun Kim***, Wee Hyun Park****, Jae Eun Jun****, Shung Chull Chae****, Kyung Eun Song*****, Sung Hee Cho*** and Hee Sook Oh***

Department of Culinary Science, Honam University, Kwangju 506-714, Korea
**Department of Preventive Medicine and Public Health, School of Medicine. Kyungpook National University, Taegu 702-701, Korea*
***Department of Food and Nutrition, Taegu University, Kyungpook 712-714, Korea*
****Department of Food and Nutrition, Catholic University of Taegu, Kyungpook 712-702, Korea*
*****Department of Internal Medicine, School of Medicine, Kyungpook National University, Taegu 702-701, Korea*
******Department of Clinical Pathology, School of Medicine, Kyungpook National University, Taegu 702-701, Korea*

Abstract

This study was carried out to evaluate whether antioxidant nutrient supplementation with α-tocopherol, vitamin C, β-carotene, and selenium reduces the lipid peroxide levels and increases the antioxidative enzyme activities in patients with coronary heart disease. Eighty nine patients participated in a randomized, double-blind, placebo-controlled trial. The antioxidant group (45 patients) was given daily doses of α-tocopherol (400 IU), vitamin C (50 mg), β-carotene (15 mg), and selenium (50 μg) and forty four patients received a placebo. Thirty eight subjects (84.4%) of the antioxidant group and thirty nine subjects (88.6%) of the placebo group completed the three-month supplementation. Serum levels of tocopherol, vitamin C and β-carotene significantly increased in the antioxidant group compared with the baseline ($p<0.05$). Thiobarbituric acid-reactive substances (TBARS) decreased significantly (0.6 nmol MDA/mL) in the antioxidant group compared with that (0.09 nmol MDA/mL) in the placebo group ($p=0.03$). However, antioxidant supplementation did not affect the level of oxidized-LDL measured as autoantibodies against oxidized-LDL. The superoxide dimutase activity in red blood cells increased in the antioxidant group compared with the baseline ($p<0.05$). However, glutathione peroxidase activities did not change after supplementation in both groups, and catalase activity significantly decreased in the placebo group ($p<0.05$). These results suggest that antioxidant supplementation for 3 months with α-tocopherol, vitamin C, β-carotene and selenium in patients with coronary heart disease may be partially protective against oxidative stress.

Key words: antioxidant nutrient, coronary heart disease, lipid peroxidation, antioxidative enzyme, randomized controlled clinical trial

INTRODUCTION

Coronary heart disease (CHD) is a leading contributor of premature death among adults in industrialized countries and developing countries. Approximately 5.4 million individuals are diagnosed with CHD annually, but the incidence of death due to CHD has declined substantially over the past 25 years by control programs aimed at reducing risk factors (1,2). In contrast, CHD mortality in Korea has been increasing over the last 10 years, so we need to develop an effective program for prevention and treatment of CHD (3).

Hypercholestrolemia, cigarette smoking, hypertension, and obesity are well known contributing risk factors for the development of atherosclerotic CHD. However, they account for only half of all causes of CHD, and the complete pathologic process underlying atherosclerosis remains unknown (4).

An important event in the pathogenesis of atherosclerosis is believed to be the oxidative modification of low density lipoprotein initiated by a free radical-driven lipid peroxidation. Therefore, current research has focused on inhibiting oxidation of LDL as a means of protection against atherosclerosis. One such approach is to enhance the endogenous antioxidant defence systems within the LDL particle with lipophilic antioxidants such as α-tocopherol and β-carotene, or by supplementing the aqueous-phase antioxidant capacity with ascorbic acid. Vitamin E radicals act as prooxidants during the autoxidation of LDL (5,6). It was also shown that the shortened lag time induced by higher doses of vitamin E was restored when lipid- and water-soluble antioxidants were added simultaneously, which suggests that vitamin E radicals derived from vitamin E are subsequently reduced by vitamin C to generate vitamin E. Thus, the interaction between lipid- and water soluble antioxidants provides an important function in maintaining LDL resistance to oxidation (7).

There is ample evidence that oxidative stress is involved in the pathogenesis of atherosclerosis, endothelial dysfunction, platelet aggregation, and heart failure (5). However, the extrapolation of this evidence to use in antioxidant therapy in primary or secondary prevention of coronary heart disease cannot be justified at this time since the data are inconclusive.

†Corresponding author. E-mail: hjjoung@honam.honam.ac.kr
Phone. 82-62-940-5416, Fax: 82-62-940-5005

Previous investigations have shown that supplementation of diet with α-tocopherol or ascorbate can decrease susceptibility of LDL oxidation (8-13). Whether these effects are additive when given in combined supplementation, however, has not been determined in depth. In the study for the primary prevention effect of antioxidant vitamins for CHD incidence, Virtamo et al. (14) reported that supplementation of α-tocopherol has only a marginal effect on the incidence of fatal coronary heart disease in male smokers with no history of myocardial infarction, but β-carotene has no primary preventive effect on major coronary events (14). The secondary preventive effects of the antioxidant vitamins for the patients with CHD are also not conclusive at this time. A statistically significant decrease in the risk of non-fatal myocardial infarction in patients with CHD was observed with α-tocopherol supplementation compared to a placebo, but there was a significant increase in the risk of fatal coronary heart disease with α-tocopherol and β-carotene supplementation, and with β-carotene alone (15,16).

Therefore, this study was carried out to ascertain that combined antioxidant supplementation with α-tocopherol, β-carotene, and selenium can lead to positive effects on antioxidant vitamin status, antioxidant enzyme activities, and lipid peroxidation to decrease the risk of coronary heart diseases.

MATERIALS AND METHODS

Subjects

Patients with coronary heart disease were recruited at a University hospital in Taegu. Inclusion criteria were at least 3 months after surgery or onset of angiographically confirmed angina pectoris and attack of myocardial infarction and age, 40~70 years. The eighty nine subjects were randomly assigned to either the supplemented group (n=45) or the age ($\pm$5 years) and sex matched placebo group (n=44), and the 77 subjects completed 3 months supplementation. The reasons for withdrawal were death (n=1), gastrointestinal trouble (n=2), and unwillingness (n=9).

Study protocol

This was a double blind, randomized placebo-controlled trial. The subjects were interviewed about their general background, smoking, exercise, alcohol consumption, and medical histories, including a question on previous angina pectoris and myocardial infarction diagnosed by a physician. Medical records were also reviewed to get information about the current treatment, complication, and confirmation of disease status. Dietary nutrient intakes were investigated for a day by the 24-hour recall method with real size photographs of various foods in order to recall the exact weight.

After random allocation they received capsules for three months. The supplements consisted of antioxidant capsules containing α-tocopherol (400 IU), β-carotene (15 mg), vitamin C (500 mg), and selenium (50 μg) (Ildong Pharmacological Inc., Seoul). Subjects were asked to take one capsule daily and keep their diet and life style during study. Placebo capsules were identical in appearance and usage. Capsules were dispensed for each 2 week period. During each visit, once every 2 weeks, subjects confirmed their usage and received additional capsules.

Blood was obtained to determine the antioxidant vitamin status, antioxidant enzyme activities, and lipid peroxidation at fasting status before and after supplementation. The red blood cells, plasma, and serum were isolated within 2 hours after blood withdrawal. The red blood cells were rinsed twice with saline solution, and a portion of serum were treated with 10% metaphosphoric acid right after isolation for vitamin C analysis. All materials were kept -80°C until assay.

Measurements

The concentration of α-tocopherol, β-carotene, and ascorbate in serum was measured, following extraction, by high performance liquid chromatography (17,18). The serum lipid peroxide concentrations were measured indirectly as the levels of thiobarbituric acid substance (TBARS) by the procedure presented by Yagi (19) and autoantibodies of oxidized LDL by kits (OLAB, Biomedica Co.). The activity of superoxide dismutase in red blood cells were analyzed by pyrogallol oxidation method (20), and those of glutathione peroxidase and catalase in red blood cells were measured (21-23).

Statistical analysis

Group means between the antioxidant supplemented group and the placebo group were compared by the t-test, and mean differences between before and after supplementation were analyzed by paired t-test. The differences of distribution of subjects by variables were analyzed by Chi-square test.

RESULTS AND DISCUSSION

Characteristics of subjects

The general characteristics of subjects at the baseline are summarized in Table 1. There were no significant differences between the placebo and antioxidant supplemented groups with respect to age (61.4 ± 8.1 and 60.8 ± 8.6, $p>0.05$), body mass index (BMI, kg/m^2, 23.6 ± 2.5 and 23.8 ± 2.7, $p>0.05$) and blood pressure. The distribution of subjects between sex and treatment groups was not significantly different by Chi-square test ($p>0.05$). Disease types and treatment characteristics of subjects such as percentages of patients with diabetes mellitus, hypertension, hyperlipidemia, complications, and medications such as taking lipid lowering agent, β-blocker, and calcium blocker, between antioxidant group and placebo group were not significantly different by Chi-square test ($p>0.05$).

There were no significant differences in the life styles such as alcohol consumption, smoking, regular exercise, taking nutrient supplementation, and taking healthy food between the two groups ($p>0.05$). The percentages of current alcohol users and smokers were higher, albeit non-significant, in the antioxidant group than that of the placebo group.

The mean daily nutrient intakes before and after supple-

Table 1. General characteristics of study subjects at the baseline[1)]

	Placebo (n=38)	Antioxidant (n=39)
Demographics/habits		
Age	61.4±8.1 years	60.8±8.6 years
Sex (male)	29(76.3)	30(76.9)
Current smoker	11(29.0)	12(30.8)
Current alcohol user	18(47.4)	12(30.8)
Regular exercise	12(31.6)	18(46.2)
Disease		
Old myocardial infarction	17(46.0)	20(51.3)
Angina pectories	21(55.3)	19(48.7)
Diabetes mellitus	8(21.1)	4(10.2)
Hypertension	14(36.8)	10(25.6)
Hyperlipidemia	16(42.1)	15(38.4)
Medications		
Lipid lowering	15(39.5)	18(46.1)
Multivitamins	8(21.0)	6(15.4)
Healthy food	8(21.0)	5(12.8)
β-Blocker	26(68.4)	30(76.9)
Calcium blocker	22(57.9)	26(66.8)
Nitrate	29(76.3)	22(56.4)
Physical examination		
Body mass index	23.6± 2.5 kg/m^2	23.8± 2.7 kg/m^2
Systolic BP	125.8±16.4 mm Hg	118.8±14.9 mm Hg
Diastolic BP	70.4±12.5 mm Hg	71.1±10.5 mm Hg

[1)]All values are number of patients (percentages) except where indicated.
Not significant for all comparisons ($p>0.05$)

mentation in the antioxidant group and placebo group are presented in Table 2. The macronutrients, such as energy, protein, fat, and β-carotene, and vitamin C were not significantly different before and after in both groups.

Thus, these same conditions at baseline between the two groups were established so as to explain the effect of antioxidant supplementation on the patients with coronary heart disease.

Effects of antioxidant supplementation

This study focused on whether antioxidant supplementation for 3 months can result in an increase of antioxidant nutrient status and antioxidant enzyme activities and decrease of lipid peroxidation to protect oxidative damage so that a secondary occurrence of coronary heart disease may be prevented. Plasma levels of all three micronutrient antioxidants were not statistically different between the two groups at the baseline and there were no significant changes in levels in the placebo group after supplementation (Fig. 1). Levels of all three antioxidants significantly increased in the antioxidant group after supplementation ($p<0.05$). Levels of serum α-tocopherol, ascorbate, and β-carotene were 2.1, 1.4, 2.0-fold higher in the supplemented group after 3 months compared with the baseline. Jialal and Grundy (24) also reported that 3 months combined supplementation with α-tocopherol, ascorbate, and β-carotene resulted in significant increases in plasma ascorbate, lipid standardized α-tocopherol, and β-carotene levels (2.6, 4.1, and 16.3-fold respectively). The reason why they found more effects on antioxidant nutrient levels might be that they supplemented with higher doses of vitamins than we used, such as supplementation with ascorbate (1.0 g/d),

Table 2. Daily mean dietary nutrient intakes of study subjects (Unit : Mean±SD)

Dietary nutrient intake	Antioxidant (n=38)		Placebo (n=39)	
	Baseline	After treatment	Baseline	After treatment
Energy (kcal)	1458.7±490.5	1454.1±538.4	1523.3±788.7	1449.9±589.8
Protein (g)	63.0±31.0	60.6±27.9	69.6±38.9	61.6±31.8
Fat (g)	32.4±19.0	33.3±22.7	32.7±19.4	31.3±24.8
Fiber (g)	6.3±4.3	6.2±5.4	7.0±5.7	5.3±2.4
Vitamin A (RE)	611.7±760.6	736.9±1014.2	559.8±452.4	702.0±697.3
Retinol (μg)	56.7±78.7	47.1±73.9	88.2±242.6	118.3±450.2
β-carotene (μg)	3210.8±4557.9	3007.0±3846.8	2762.6±2226.4	3266.7±3367.7
Vitamin C (mg)	90.6±64.7	86.9±60.0	100.0±71 4	86 0±41.0

$p>0.05$ for all comparisons of mean daily nutrients intakes between baseline and after treatment in both groups at paired t-test, and between antioxidant and placebo in baseline at t-test.

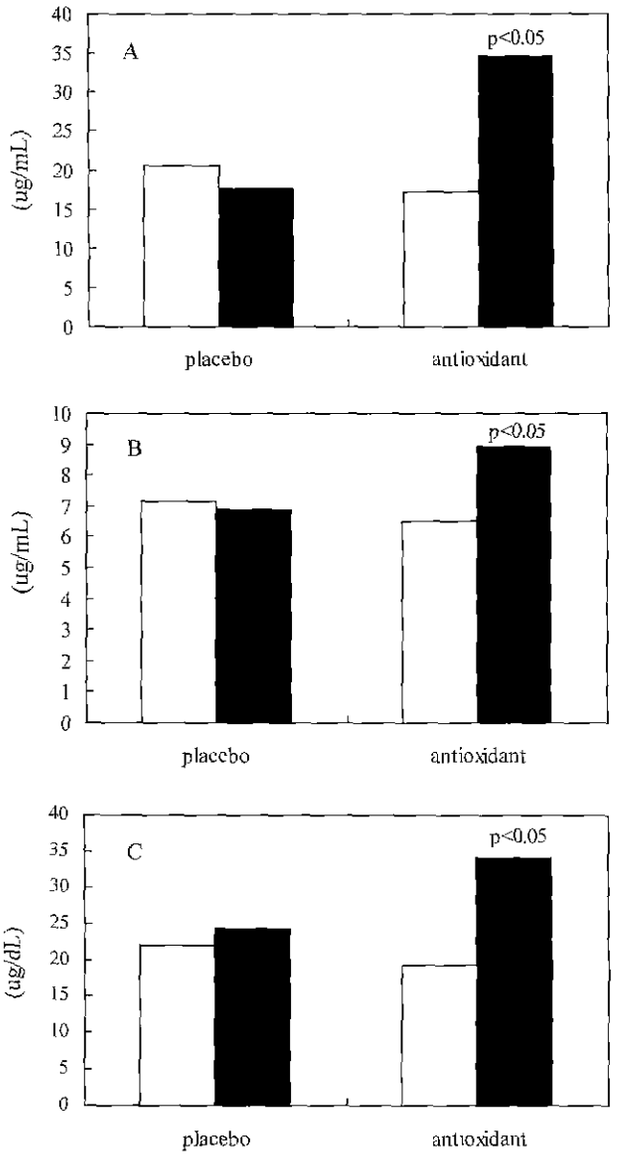

Fig. 1. Serum levels of ascorbate, α-tocopherol, and β-carotene in the placebo and antioxidant supplemented groups. P-value means significant mean changes of antioxidant vitamin levels compared with the baseline by paired t-test. □, baseline ; ■, after treatment. A, alpha-tocopherol ; B, ascorbate ; C, beta-carotene.

β-carotene (30 mg/d), and α-tocopherol (800 IU/d). Further research about the dose-response relationship between the amount of antioxidants and effects in these conditions may be required for explanation.

In healthy conditions, there are delicate balances between free radicals and antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), vitamin E, and vitamin C. These antioxidants partially reduce oxygen free radicals and oxidative stress to prevent various degrees of cellular damage. However, under pathological conditions, the balance may be tilted toward the oxidative side and result in cellular damage such as lipid peroxidation, DNA chain breaking, and enzyme inactivation (25). Previous studies reported that patients with coronary heart disease have had decreased antioxidant enzyme activities, and antioxidant nutrient supplementation could increase these enzyme activities (26,27).

In order to see whether these increases of antioxidant status in serum can decrease susceptibility of oxidative stress, the antioxidant enzyme activities and lipid peroxides were measured after 3 months supplementation. In this study, SOD and catalse activities of red blood cells were not different between the two groups at the baseline, but GPX activity of RBCs in antioxidant group were higher than in placebo group ($p< 0.05$). The SOD activity in RBCs was increased significantly at 3 months in the antioxidant supplemented group compared with the baseline ($p<0.05$), but not in the placebo group (Fig. 2). There were no significant differences in activities of CAT and GPX in the antioxidant supplementation group compared with the baseline, however, catalase activity in placebo significantly decreased at 3 months compared with the baseline ($p<0.05$) (Fig. 2). Further research about the reasons are required.

On the other hand, increases in antioxidant vitamin status and SOD activity could partially lead to a decrease in lipid peroxidation (Fig. 3). The concentrations of TBARS was 25% decreased at 3 months from the baseline (2.4 ± 1.4 nmol MDA/mL and 1.9 ± 0.9 nmol MDA/mL) in the antioxidant supplemented group, but not in the placebo. However, the level of oxidized LDL measured by autoantibodies of oxidized LDL did not change after supplementation in both groups compared with the baseline. In this case, we need more research on the reasons because autoantibodies of oxidized LDL or TBARS may not be sensitive indicators of the short term effects of antioxidant supplementation or duration or dosage of supplementation was not sufficient to cause an increase in autoantibodies of oxidized LDL. Hoffman and Garewal (28) reported when the 33 subjects with coronary heart disease were treated 400 mg/day of α-tocopherol for 6 months, plasma α-tocopherol significantly increased, but oxidized LDL measured by formation of thiobarbituric acid-reactive substance (TBARS) did not change significantly. The other study also showed that when the 20 patients attending a lipid clinic were treated with 100 IU vitamin E daily and doubled at six-weekly intervals to 1600 IU daily, a significant increase in both α-tocopherol levels and the lengths of lag phase was seen in the vitamin E group after the first week of supplementation (100 IU/day) and this continued to rise in a dose dependent fashion with a doubling of the lag phase at 1600 IU daily. However, the titre of antibodies to malondialdehyde (MDA) derivatized-LDL was not altered (9). In addition, α-tocopherol supplementation (450 IU daily) in twenty-eight men with verified coronary heart disease and hypercholesterolemia led to a 1.9-fold concentration of reduced α-tocopherol in LDL, prolonged depletion time 100.9%, and 43% prolongation was seen in the lag time of conjugated-diene formation (8). Thus, the titre of antibodies to oxidized-LDL may not be a suitable indicator to evaluate the effect of short-term antioxidant supplementation.

In conclusion, the results of present study imply that combined antioxidant supplementation with α-tocopherol (400

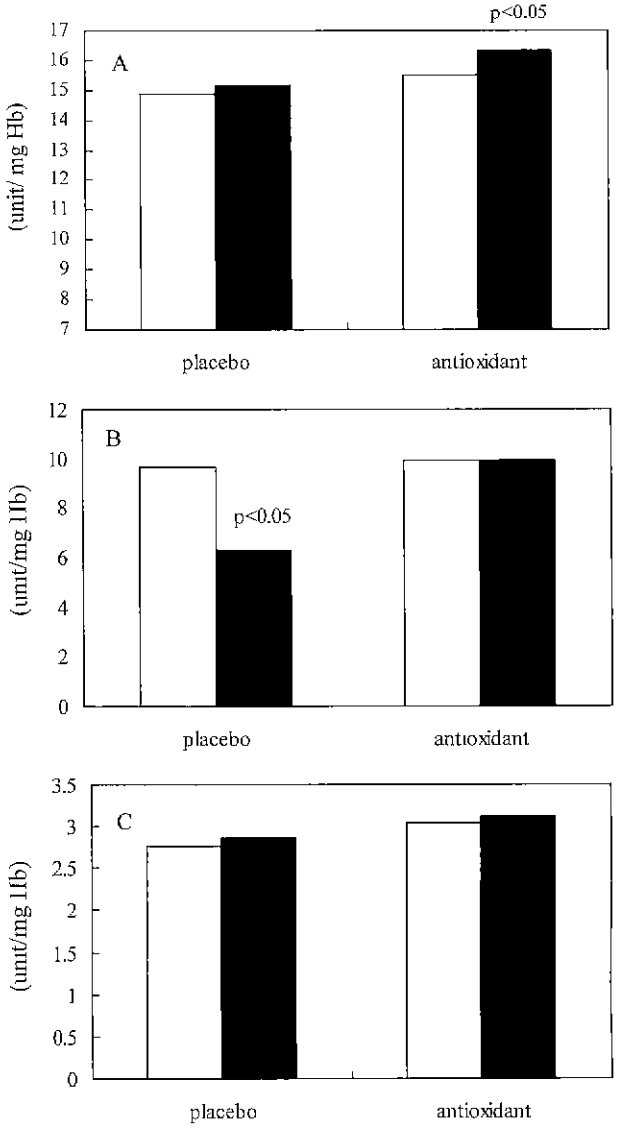

Fig. 2. Effects of antioxidant supplementation to antioxidant enzyme activities in red blood cells. P-value means significant changes of antioxidant enzyme activities compared with the baseline by paired t-test. □, baseline ; ■, after treatment. A, superoxide dismutase ; B, catalase ; C, glutathione peroxidase.

IU), β-carotene (15 mg), vitamin C (500 mg), and selenium (50 μg) for 3 months could lead partially to increase antioxidant enzyme activity and decrease concentration of lipid peroxide, but further research about antioxidant supplementation effect with various dosage, duration, and disease status are necessary.

Although oxidative stress is involved in the pathogenesis of atherosclerosis, the use of antioxidant supplementation in primary or secondary prevention of cardiovascular disease cannot be justified at this time (5). In the randomized, placebo-controlled, double blind study with a large population, the results of supplementation effects with vitamin E, ascorbate, and β-carotene were not consistent. Rapola et al. (15) reported that there was no significant effect on the total number of major coronary events in any of the supplementation groups.

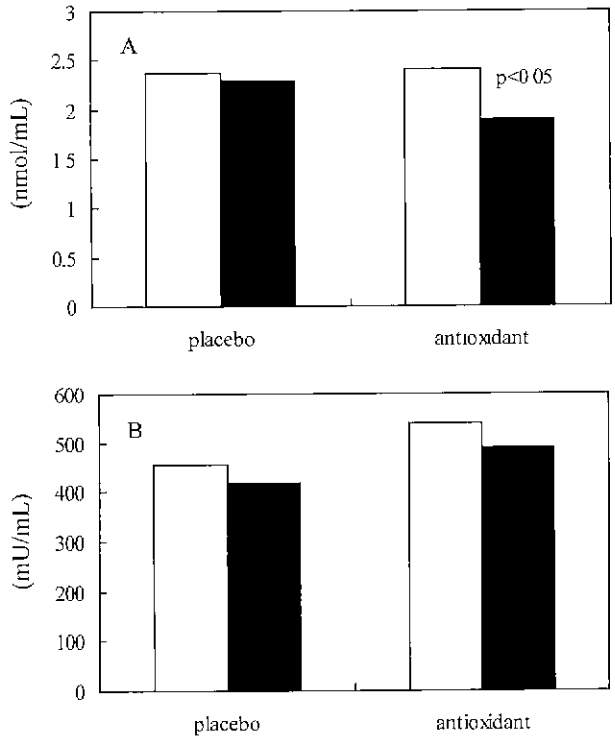

Fig. 3. Mean concentrations of TBARS and autoantibodies of oxidized LDL at the baseline and after treatment in two groups. P-value means significant change of lipid peroxide concentration compared with the baseline by paired t-test. □, baseline ; ■, after treatment. A, TBARS ; B, autoantibodies of oxidized LDL.

The Cambridge Heart Antioxidant Study (CHAOS) also showed that alpha-tocopherol decreased the risk of the non-fatal myocardial infarction by 77%, but there was an 18% increase of cardiovascular deaths with alpha-tocopherol. Therefore, the research on antioxidant supplementation effects on the incidence or mortality of coronary artery heart disease patients is also required to set the strategy for primary and secondary prevention of coronary artery disease in Korea.

REFERENCES

1. WHO MONICA Project Principal Investigators. The World Health Organization MONICA Project (Monitoring trends and determinants in cardiovascular diseases): geographic variation in mortality from cardiovascular disease-baseline data on selected population characteristics and cardiovascular mortality. *World Health Stat Q*, **40**, 171 (1987)
2. McGovern, P.G., Pankow, J.S., Shamar, E., Doliszny, K.M., Folson, A.R. and Blacksburn, H. . Recent trends in acute coronary heart disease, mortality, morbidity, medical care, and risk factors. *N. Engl. J. Med.*, **334**, 884 (1996)
3. National statistical office republic of Korea : Annual report on the cause of death statistics (Based on vital registration) (1998)
4. Blackburn, H. and Luepker, R. : Heart disease. In "*Public health and preventive medicine*" Last, J.M. (ed.), Appleton-Century-Crofts, p.1159 (1986)
5. Hoeschen, R.J. : Oxidative stress and cardiovascular disease. *Can. J. Cardiol.*, **13**, 1021 (1997)
6. Rapola, J.M. : Should we prescribe antioxidants to patients with

coronary heart disease? *Eur. Heart J.*, **19**, 530 (1998)
7. Hirano, R., Kondo, K., Iwamoto, T., Igarashi, O. and Itakura, H. : Effects of antioxidants on the oxidative susceptibility of low-density lipoprotein. *J. Nutr. Sci. Vitaminol.*, **43**, 435 (1997)
8. Palomaki, A., Malminiemi, K., Malminiemi, O. and Solakivi, T. : Effects of Lavastatin therapy on susceptibility of LDL to oxidation during α-tocopherol therapy. *Arterioscler Thromb Vasc. Biol.*, **19**, 1541 (1999)
9. Wen, Y., Killalea, S., Norris, L.A., Cooke, T. and Feely, J. : Vitamin E supplementation in hyperlipidemic patients : effect of increasing doses on *in vitro* and *in vivo* low-density lipoprotein oxidation. *Eur. J. Clin. Invest.*, **29**, 1027 (1999)
10. Princen, H.M.G., Van Poppel, G., Vogelezang, C., Bluytenhak, R. and Kok, F.J. : Supplementation of vitamin E but beta carotene in vivo protects LDL from lipid peroxidation *in vitro*. *Arterioscler. Thromb.*, **12**, 554 (1992)
11. Esterbauer, H., Gebicki, J., Puhl, H. and Jurgens, G. : The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Rad. Biol. Med.*, **13**, 341 (1992)
12. Esterbauer, H., Dieber-Rotheneder, M., Striegl, G. and Waeg, G. : Role of vitamin E in preventing the oxidation of low density lipoprotein. *Am. J. Clin. Nutr.*, **53**, 314S (1991)
13. Dieber-Rotheneder, M., Puhl, H., Waeg, G., Striegl, G. and Esterbauer, H. : Effect of oral supplementation with d-alpha-tocopherol on the vitamin E content of human low density lipoprotein and resistance to oxidation. *J. Lipid Res.*, **32**, 1325 (1991)
14. Virtamo, J., Rapola, J.M., Rioatti, S., Heinonen, O.P., Taylor, P.R. and Albanes, D. : Effect of vitamin E and beta carotene in the incidence of primary nonfatal myocardial infarction and fetal coronary heart disease. *Arch. Intern. Med.*, **158**, 668 (1998)
15. Rapola, J.M., Virtamo, J., Ripatti, S., Huttunen, J.K., Albanes, D. and Taylor, P.R. : Randomized trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infraction. *Lancet*, **349**, 1715 (1997)
16. Stephen, N.G., Parsons, A., Schofield, P.M., Kelly, F., Cheeseman, K., Mitchinson, M.J. and Brown, M.J. : Randomized controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet*, **347**, 781 (1996)
17. Bieri, G., Tolliver, J.J. and Catignani, G.L. : Simultaneous determination of alpha-tocopherol and retinol in plasma or red blood cells by high pressure liquid chromatography. *Am. J. Clin. Nutr.*, **32**, 2143 (1979)
18. Otsuda, M., Kurota, T., Suzuki, E., Arakawa, N. and Inagaki, C. : Separative determination of ascorbic acid and erythrobic acid in animal tissues by high performance liquid chromatography. *J. Nutr. Sci. Vitaminol.*, **27**, 9 (1981)
19. Yagi, K. : A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem. Med.*, **15**, 212 (1976)
20. Prohaska, J.R : Changes in tissue growth, concentrations of copper, iron, cytochrome c oxidase and superoxide dismutase subsequent to dietary or genetic copper deficiency in mice. *J. Nutr.*, **113**, 2148 (1983)
21. Paglia, D.E. and Valentine, W.N. : Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidation. *J. Lab. Clin. Med.*, **70**, 158 (1967)
22. Deagen, J.T., Butler, J.A., Beilstein, M.A. and Whagner, P.D. : Effects of dietary selenite, selenosystein and selenomethione O-selenocystein lyase and glutathione peroxidase activities and selenium levels in rat tissues. *J. Nutr.*, **117**, 91 (1987)
23 Luck, H. : Catalase. In "*Methods of Enzymatic Analysis*" Bergmeyer, H.U. (ed.), 2nd ed., Academic press, New York and London, p.885 (1963)
24. Jialal, I. and Grundy, S.M. : Effect of combined supplementation with α-tocophrol, ascorbate, and beta-carotene on low-density lipoprotein oxidation. *Circulation*, **88**, 2780 (1993)
25. McCord, J.M. : Human disease, free radicals and oxidant/antioxidant balance. *Clin. Biochem.*, **26**, 351 (1993)
26. Buczynski, W., Wachowicz, K., Kedziora-Kornaatowska, K., Tkaczewski, W. and Kedziora, J. : Changes in antioxidant enzymes activities, aggregability and malonyldialdehyde concentration in blood platelets from patients with coronary heart disease. *Atherosclerosis*, **100**, 223 (1993)
27. Salonen, J.T., Salonen, R. and Seppanen, K. : Effects of antioxidant supplementation on platelet function : randomized pair-matched, placebo-controlled, double-blind trial in men with low antioxidant status. *Am. J. Clin. Nutr.*, **53**, 1222 (1991)
28. Hoffman, R.M. and Garewal, H.S. : α-tocopherol supplementation for men with existing coronary artery disease : A feasibility study. *Preventive Med.*, **29**, 112 (1999)

(Received December 27, 2000)